The Army's University Affiliated Research Center

The UTEXAS UARC
Chem-Bio Program Objectives

Dr. Steve Kornguth, Director
Biological and Chemical (B/C) Countermeasures

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Simultaneous Detection of Multiple Pathogenicity Islands (PI)

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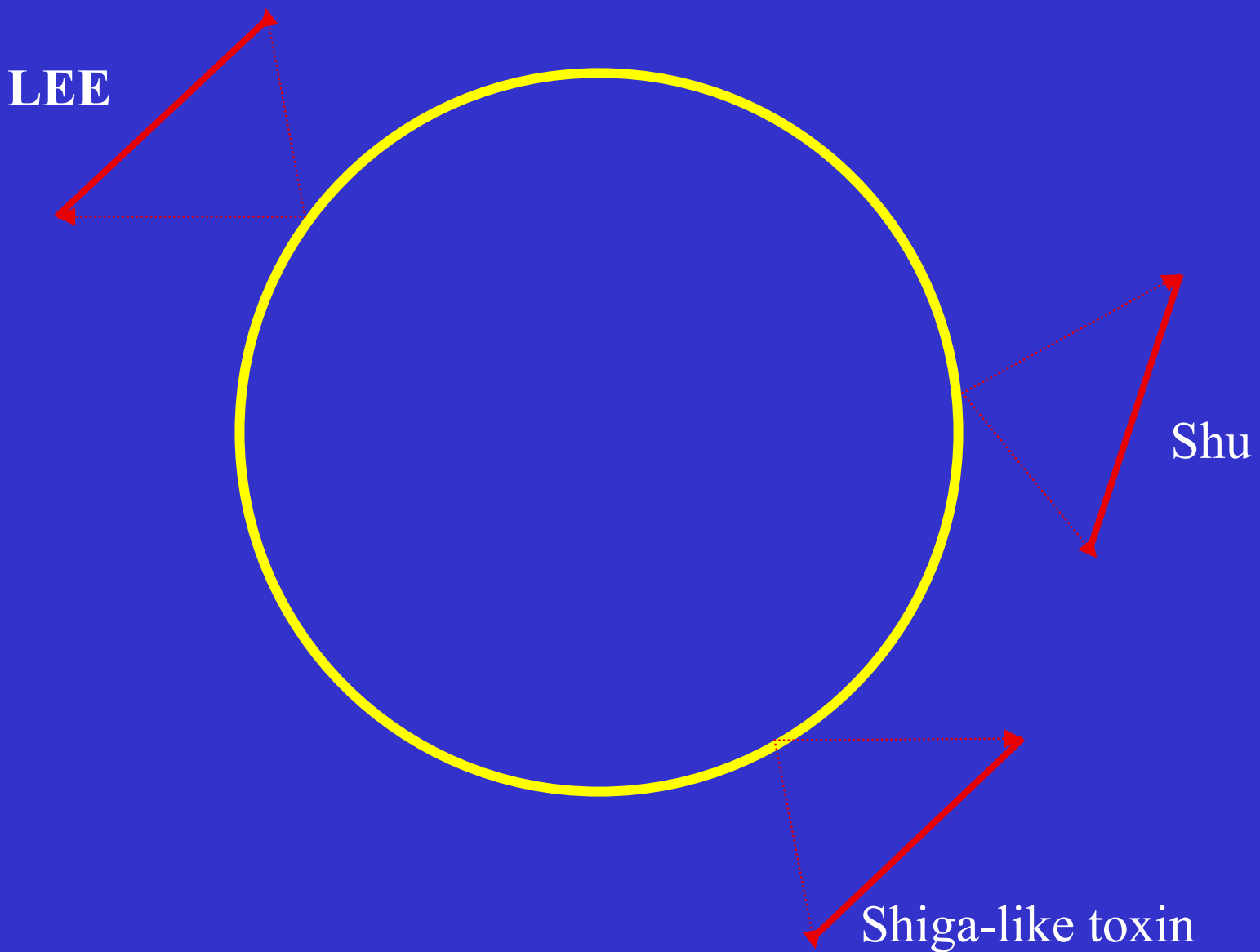
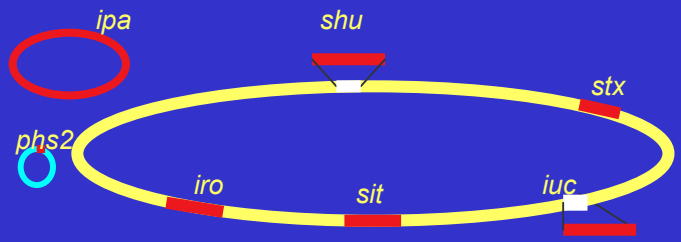
Dr. Steve Kornguth, Dr. Robert C. Chin*, Institute for Advanced Technology, UT Austin

Goal

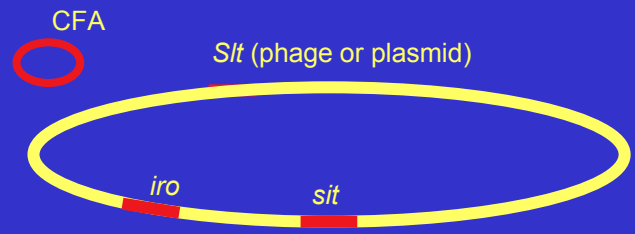
- Characterize **pathogenicity islands**, DNA factors present in virulent strains but absent from closely related, avirulent strains of bacteria
- Transition UT pathogenicity island technology to rapid commercial screening platform

E. coli O157:H7





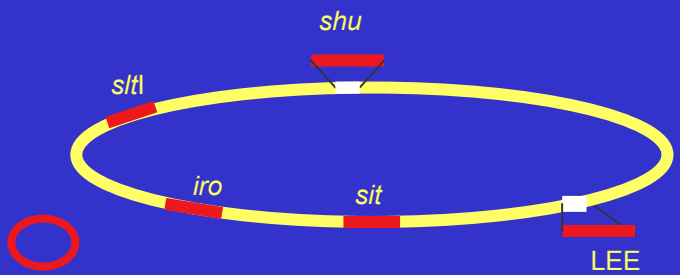
Shigella/EIEC



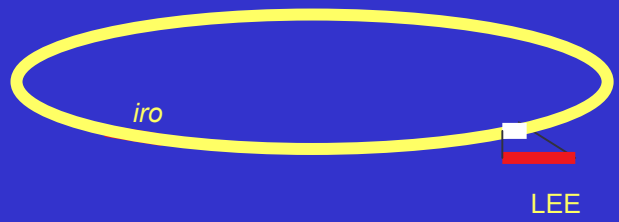
E. coli ETEC

non-pathogenic *E. coli*

E. coli O157:H7



E. coli EPEC



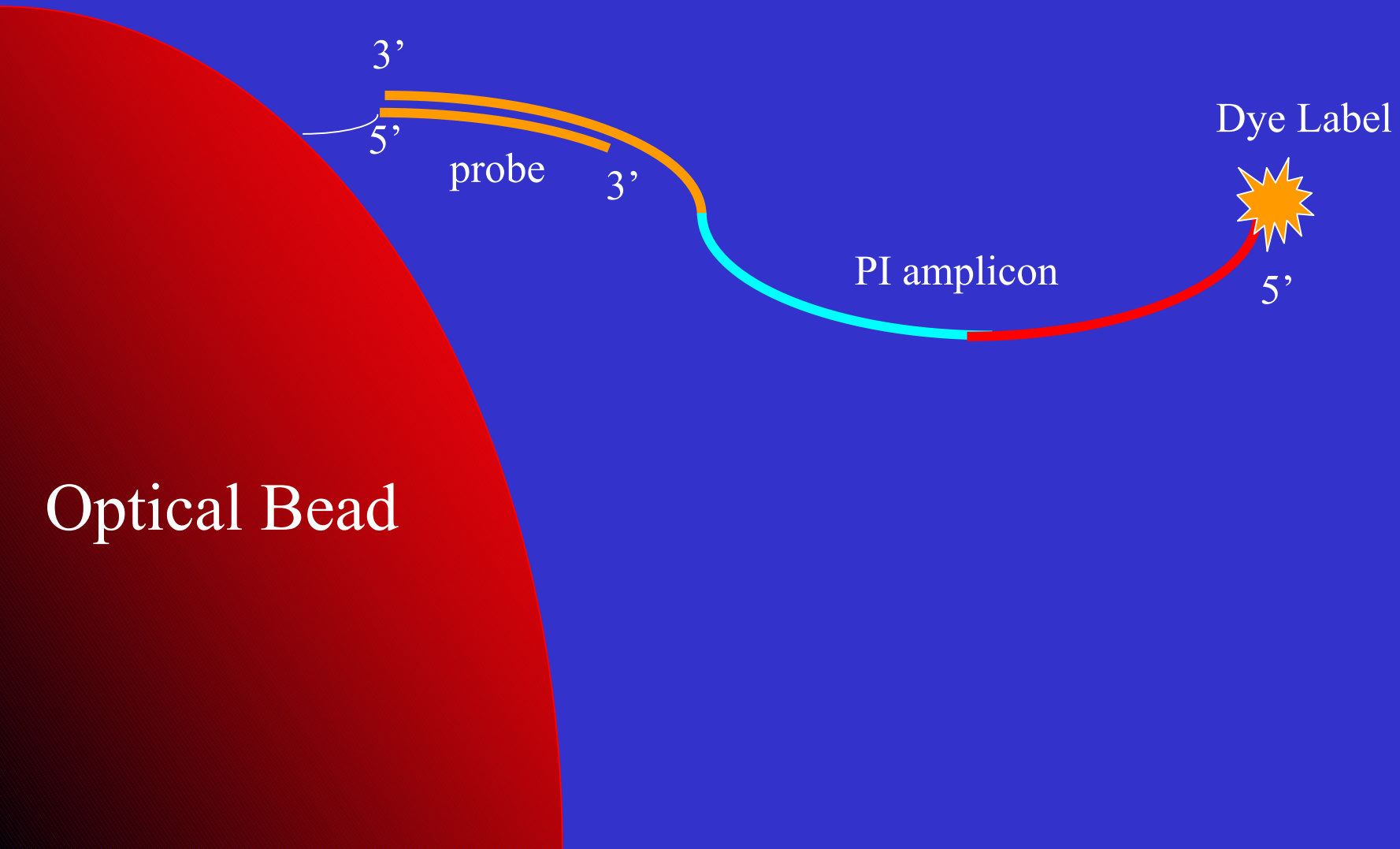
General Characteristics of a rapid screening platform

- Flexible
- Reproducible
- Simple
- Stable
- Minimize false positives and negatives

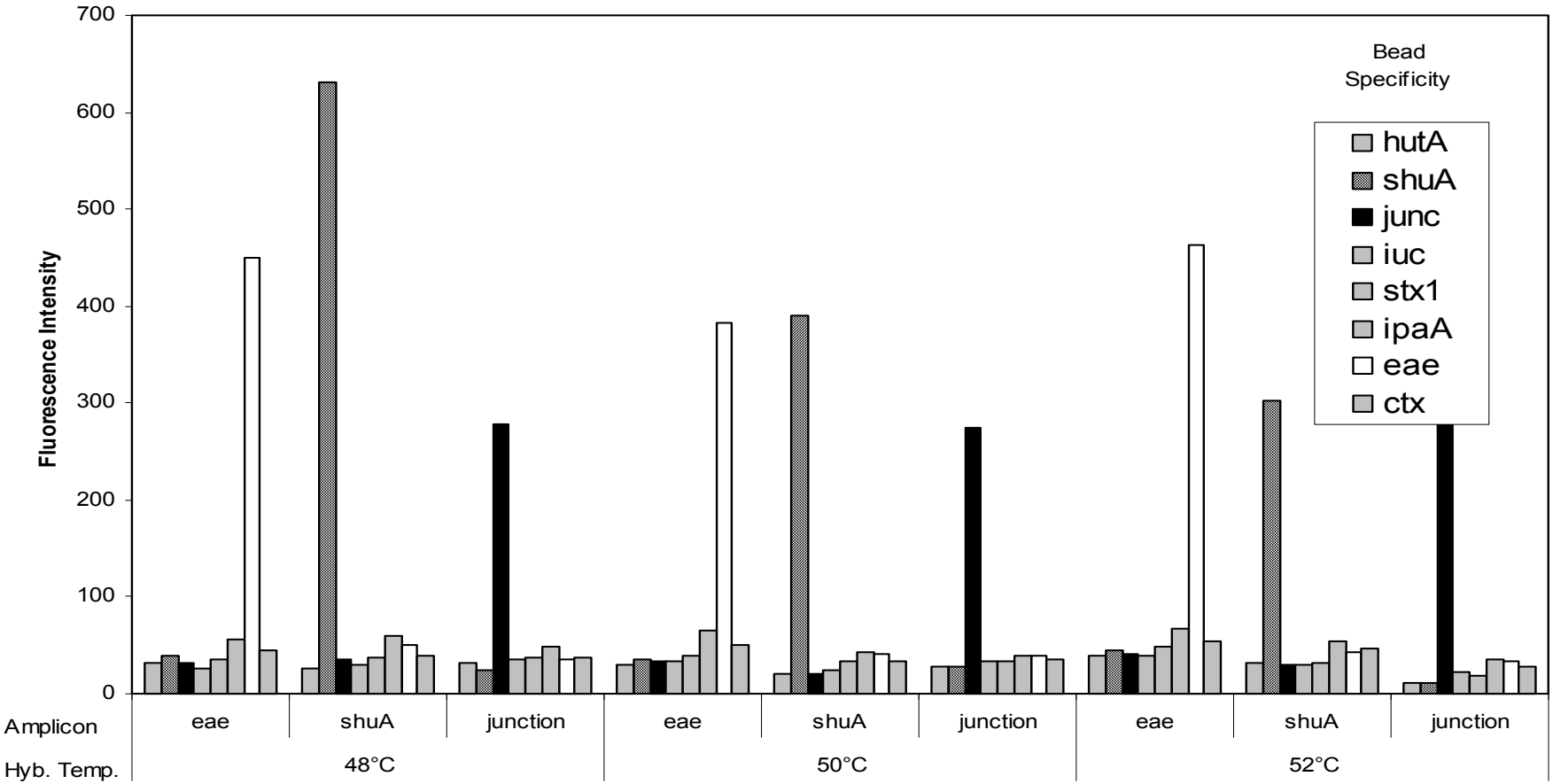
Pathogenicity Island Multiplexing

- Identify pathogenic properties rather than specific organisms
- Pattern identification of pathogens
- As new factors can be rapidly added to current stocks
- Multiple sequences for each factor can essentially eliminate false positives and false negatives

Pathogenicity Island Assay Format



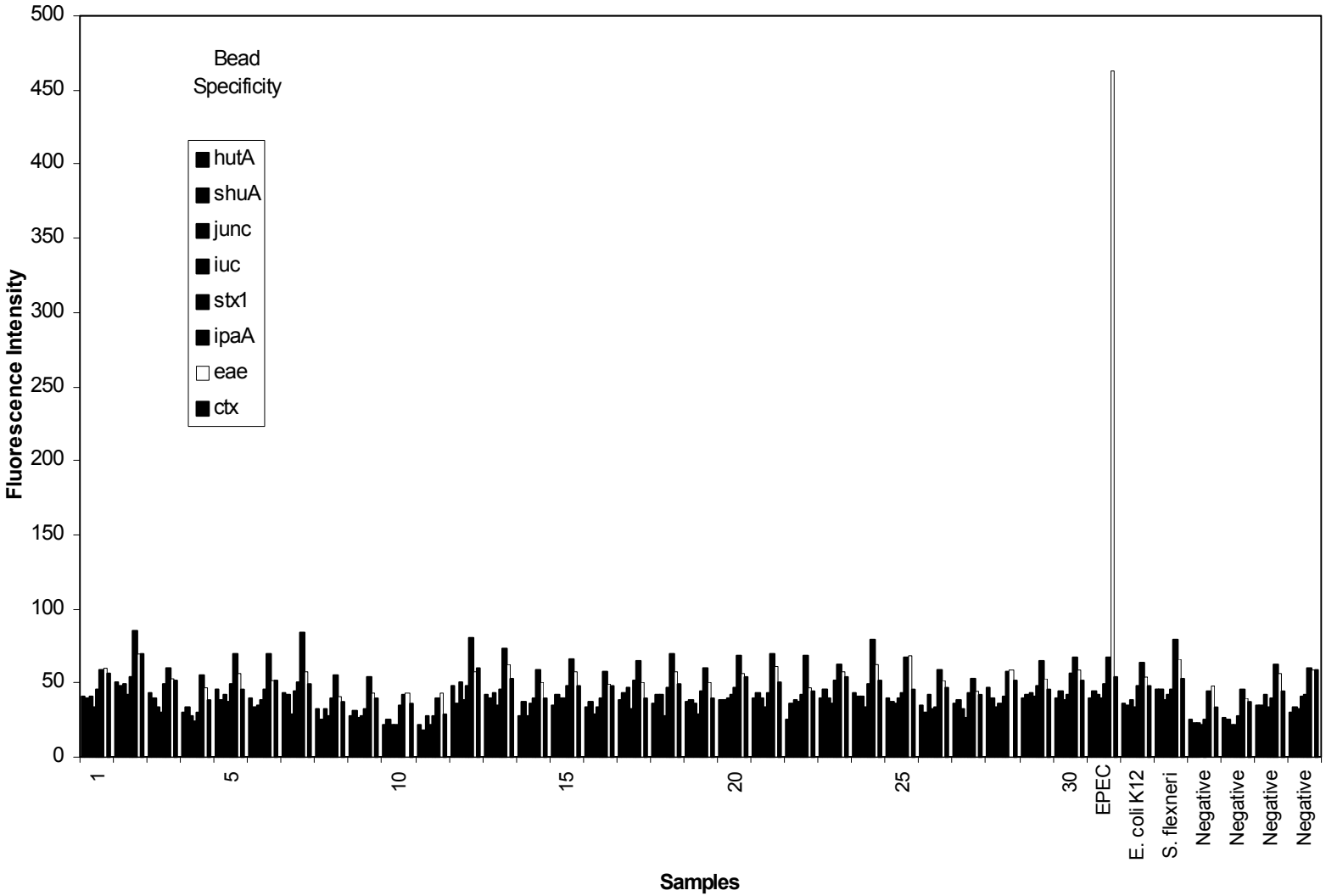
IAT/Radix Pathogenicity Island Multiplexing Assay Temperature study



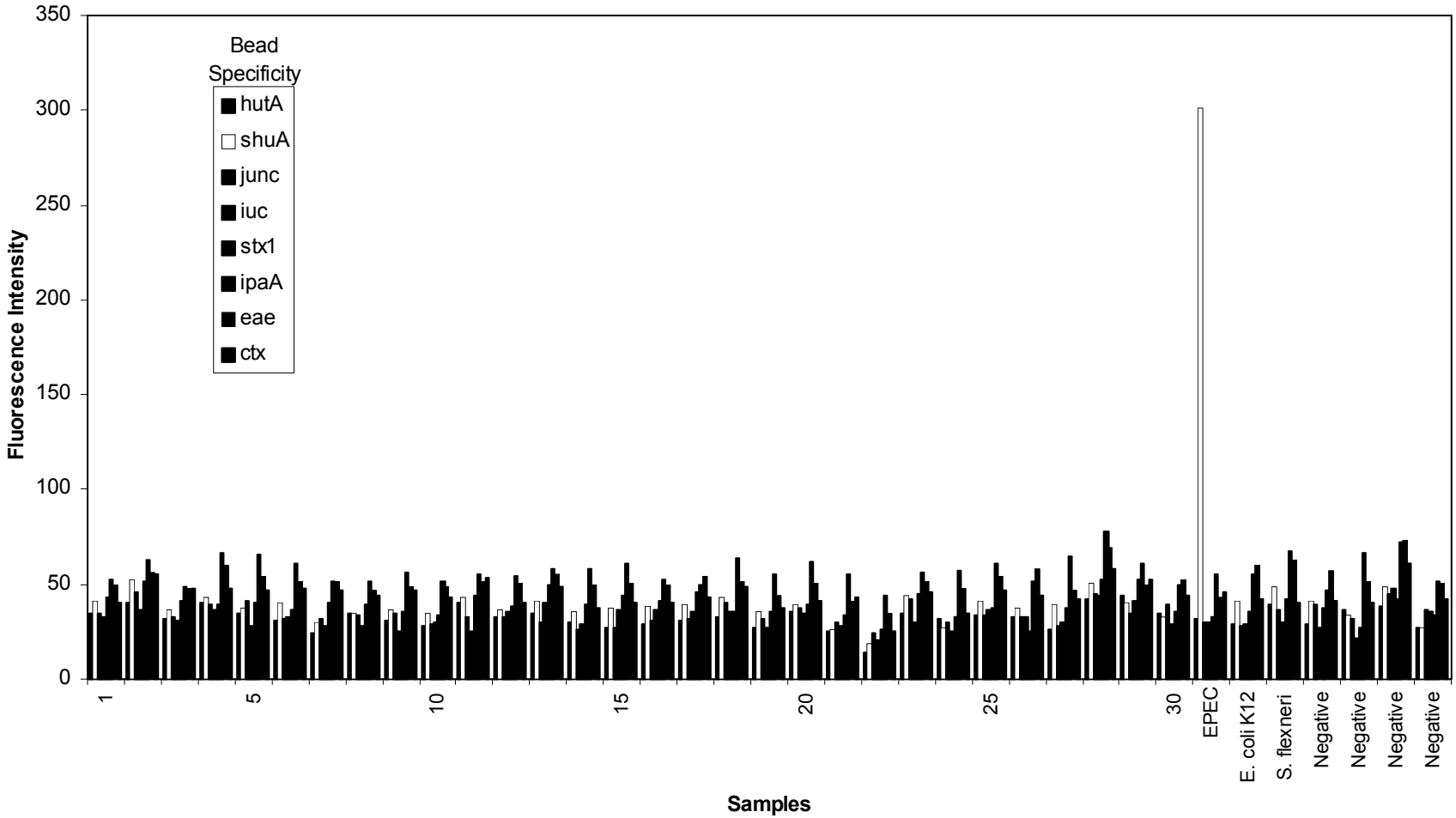
Are Pathogenicity Islands Ubiquitously Distributed in the Environment?

- 30 Dormitory rooms sampled
  - Surface swabbed (3 surfaces per room)
  - Inoculated into broth
- Amplified with 4 different PI primer sets
- Hybridized with 8 different probes at 52°C

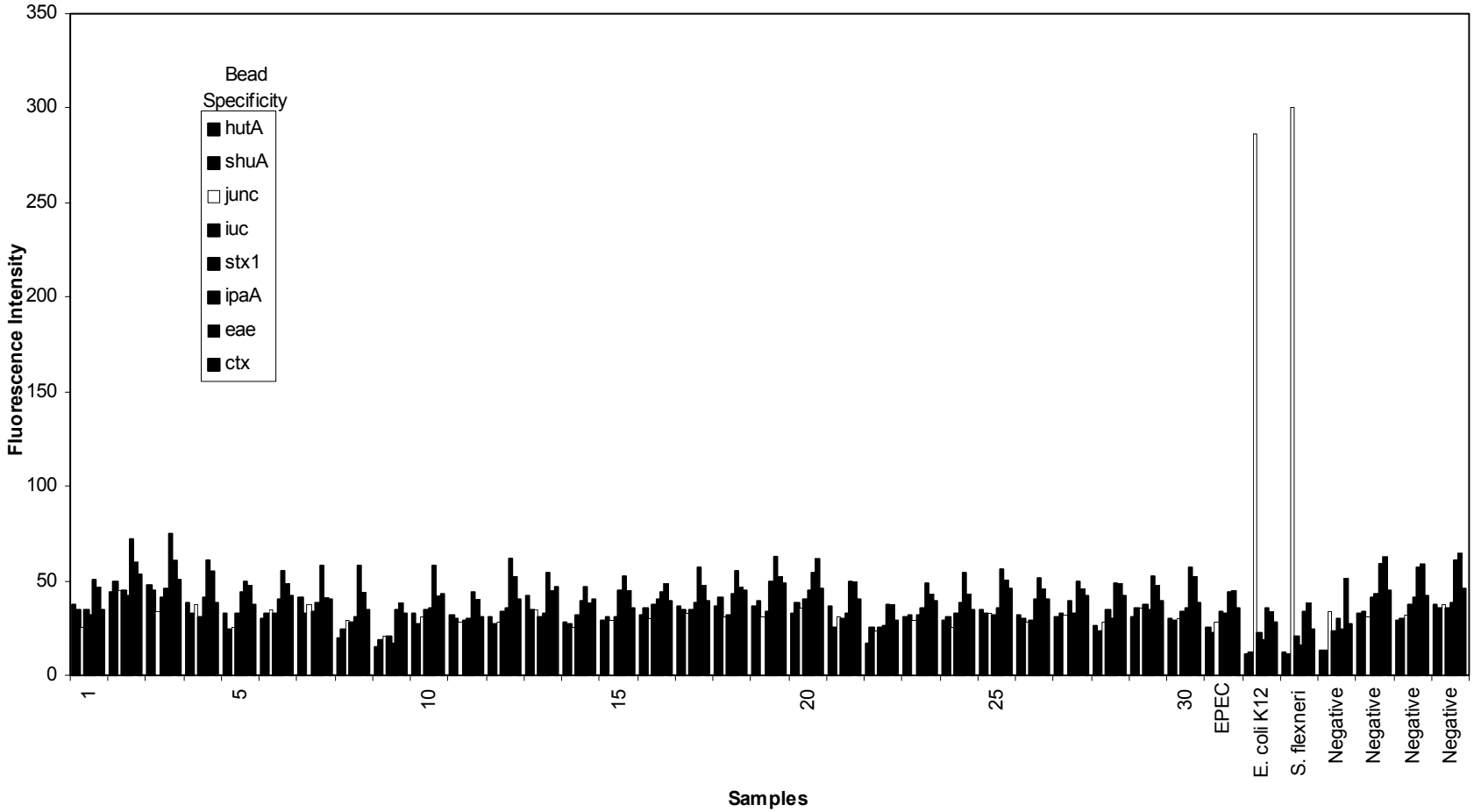
30 Dormitory room samples eae amplification



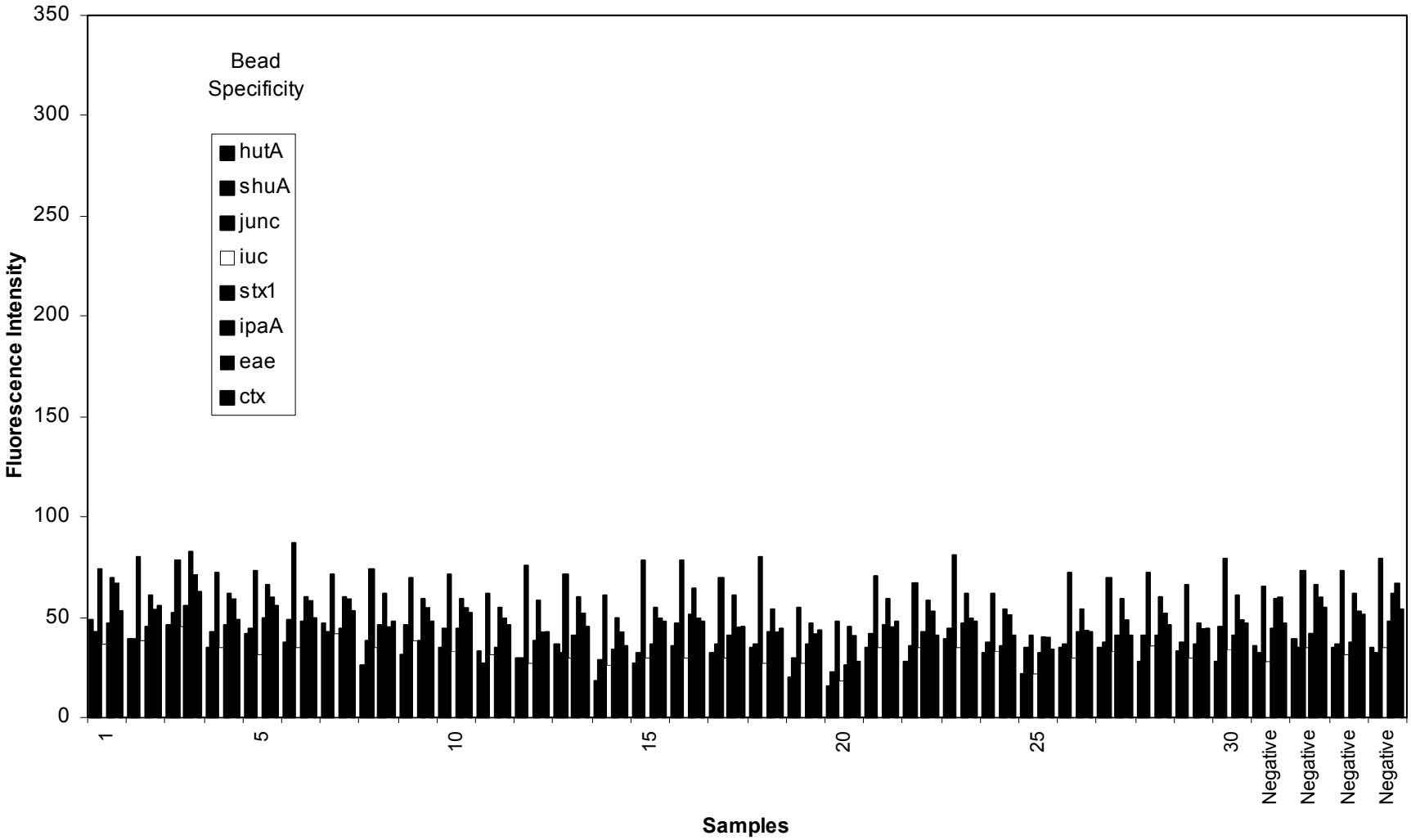
30 Dormitory room samples
Shu amplification



30 Dormitory room samples junction amplification



30 Dormitory room samples
iuc amplification

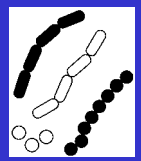


UT-Radix Detection/Identification Platform

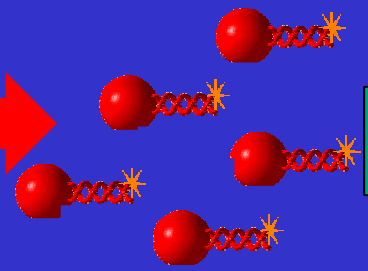
Cyclone Air Sampler

Luminex xMAP

Multiplex Pathogenicity Islands Sequence Identified



Unknown Biological Aerosol



Military Operations, Environmental Pathogens

Warning of Presence of a Virulent Organism

Civilian, Hospital, Schools
Food Borne & Office Building
Bio-surveillance Monitoring

Conclusions

- Probes have been designed to hybridize to specific PI sequences
- The PI assay has good sensitivity
- No genomic sequences for pathogens are detected in dormitory environments, therefore pathogens are NOT distributed ubiquitously in such environments

Future Plans

- Optimize probe design to eliminate cross-reactivity thus eliminating false positives and false negatives
- Optimize amplicon design needs to be undertaken to eliminate steric effects in the assay design
- Determine maximum PI amplicons that can be simultaneously screened

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